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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF INTERMITTENT CLAUDICATION OR ALZHEIMER'S DISEASE

(57) Abstract: The present invention provides methods for the treatment and prevention of intermittent claudication or Alzheimer's disease in a subject, comprising administering to a subject a formulation comprising a precursor of NO, e.g., L-arginine and a formulation comprising an agonist of eNOS, e.g., and HMG-CoA reductase inhibitor, or a formulation comprising both L-arginine and an HMG-CoA reductase inhibitor. The invention further provides that the formulations used to treat or prevent intermittent claudication or Alzheimer's disease contain at least one controlled release agent. In a further embodiment, the production of NO is substantially uniform over a prolonged period of time.

METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF INTERMITTENT CLAUDICATION OR ALZHEIMER'S DISEASE

Related Application

This application claims priority to U.S. provisional patent application Serial No. 60/379,684, filed May 9, 2002. The entire content of the aforementioned application is hereby incorporated by reference.

Background of the Invention

A family of enzymes called nitric oxide synthases (NOS) synthesize nitric oxide (NO), an important biological second messenger, from L-arginine. There are several distinct isoforms of NOS including constitutive NOS (cNOS) and inducible NOS (iNOS). There are two different kinds of cNOS: endothelial NOS (eNOS) and neuronal NOS (nNOS). eNOS is involved in the regulation of smooth muscle relaxation, blood pressure lowering, and inhibition of platelet aggregation. eNOS resides in endothelial cells and releases NO over short periods in response to receptor-mediated increases in cellular Ca^{2+}. Michel *et al.*, "Nitric oxide synthases: which, where, how, and why?," *J. Clin. Invest.*, **1997**, *100*, 2146-2152. nNOS is important for long-term potentiation, and is responsible for the Ca^{2+} dependent release from neurons. iNOS acts in host defense and is generated by activated macrophage cells during an immune response, and is induced in vascular smooth muscle cells, for example, by various cytokines, microbial products, and/or bacterial endotoxins, and once expressed, synthesizes NO for long periods of time.

Formation of nitric oxide by cNOS in endothelial cells is thought to play an important role in normal blood pressure regulation, prevention of endothelial dysfunction such as hyperlipidemia, arteriosclerosis, thrombosis, and restenosis. Functionally, cNOS, which is the predominant synthase present in brain and endothelia, is active under basal conditions and can be further stimulated by increases in intracellular calcium that occur in response to receptor-mediated agonists or calcium ionophores. cNOS appears to be the "physiological" form of the enzyme and plays a role in a diverse group of biologic processes. In vitro studies suggest that the activity of NOS can be regulated in a negative feedback manner by nitric oxide itself. In

cardiocerebrorenovascular circulation, the primary target for constitutively produced NO is believed to be soluble guanylate cyclase located in vascular smooth muscle, the myocardium (myocytes) and coronary vascular smooth muscle.

In contrast to cNOS, the inducible, calcium-independent isoform, iNOS was initially only described in macrophages. It is now known that induction of nitric oxide synthase can occur in response to appropriate stimuli in many other cell types. This induction occurs both in cells that normally do not express a constitutive form of nitric oxide synthase, such as vascular smooth muscle cells, as well as in cells such as those of the myocardium that express considerable levels of the constitutive isoform.

iNOS exhibits negligible activity under basal conditions, but in response to factors such as lipopolysaccharide and certain cytokines, expression occurs over a period of hours. The induced form of the enzyme produces much greater amounts of NO than the constitutive form, and induced NOS appears to be the "pathophysiological" form of the enzyme because high concentrations of iNOS produced NO can be toxic to cells. Induction of iNOS can be inhibited by glucocorticoids and some cytokines. Relatively little is known about post-transcriptional regulation of iNOS. Cytotoxic effects due to NO are probably largely independent of guanylate cyclase and cyclic GMP formation. Most of the research in this area has focused on the stimulation of iNOS inhibitors using various derivatives of L-arginine.

NO is a relatively stable free radical synthesized from molecular oxygen and the guanidino nitrogen of L-arginine in a reaction catalyzed by NOS. This enzyme is found in many tissues and cell types including neurons, macrophages, hepatocytes, smooth muscle cells, endothelial cells of the blood vessels, and epithelial cells of the kidney. NO acts near its point of release, entering the target cell and activates the cytosolic enzyme guanylate cyclase, which catalyzes the formation of the second messenger cGMP. Within seconds of the formation of NO, it undergoes oxidation to nitrite or nitrate. David L. Nelson, Michael M. Cox, Lehninger Principles of Biochemistry, p. 892, 3rd ed. Worth Publishers, 2000.

In response to a variety of vasoactive agents and even physical stimuli, the endothelial cells release a short-lived vasodilator called endothelium derived relaxing factor (EDRF) (also referred to as endothelium derived nitric oxide (EDNO)). Products of inflammation and platelet aggregation such as serotonin, histamine, bradykinin,

purines, and thrombin exert all or part of their action by stimulating the release of NO. Endothelial cell-dependent mechanisms of relaxation are important in a variety of vascular beds, including the coronary circulation. Hobbs, et al., "Inhibition of nitric oxide synthase as a potential therapeutic target," *Annu. Rev. Pharmacol. Toxicol.*, **1999**, *39*, 191-220. NO diffuses readily to the underlying smooth muscle and induces relaxation of vascular smooth muscle by activating guanylate cyclase, which increases cyclic GMP concentrations.

NO is responsible for the endothelium dependent relaxation and activation of soluble guanylate cyclase, neurotransmission in the central and peripheral nervous systems, and activated macrophage cytotoxicity. In the vasculature, EDNO has several actions among which are the inhibition of platelet aggregation, adhesion of inflammatory cells, and the proliferation of smooth muscle cells. In particular, EDNO is an important regulator of vascular tone. Also, flow dependent dilation, a commonly used index of endothelial function, is largely mediated by NO.

The mechanism for the regulation of vascular tone by NO is initiated by stimuli, such as acetylcholine, bradykinin, shear stress, etc., on the endothelial cells lining the vasculature. NO is produced from L-arginine through the catalytic activity of eNOS contained in these endothelial cells. The NO produced leaves the endothelial cells and stimulates the guanylate cyclase activity in the adjoining smooth muscle cells. Activation of guanylate cyclase increases the level of cGMP and causes the smooth cell to relax, thus dilating the vessel and increasing the blood flow. *See*, Moncada *et al.*, *New Eng. J. Med.*, **1993**, *329*, 2002-2012; and Vallance, *et al.*, *J. Royl. Coll. Physician London*, **1994**, *28*, 209-219.

Summary of the Invention

The present invention provides methods for the treatment and prevention of intermittent claudication (referred to herein as "IC") and Alzheimer's disease (referred to herein as "AD"). The present invention is based, at least in part, on the discovery that administering to a subject a formulation comprising an agonist of endothelial nitric oxide synthase (eNOS), such as an HMG-CoA reductase inhibitor, and a formulation comprising a precursor of NO, such as L-arginine, may be used to treat or prevent IC and AD in a subject.

Accordingly, in one aspect the invention features a method for treating a subject, *e.g.*, a human subject, comprising administering to a subject a formulation comprising a precursor of NO and a formulation comprising an agonist of eNOS, thereby treating intermittent claudication and Alzheimer's disease in a subject. In one embodiment, the progression of Alzheimer's disease is slowed. In another embodiment, the onset of Alzheimer's disease is delayed. In another embodiment, either the formulation comprising L-arginine or the formulation comprising an HMG-CoA reductase inhibitor, or both, are in tablet form.

In one embodiment, the precursor of NO is L-arginine and the agonist of eNOS is an HMG-CoA reductase inhibitor.

In another aspect, the invention features a method of treating intermittent claudication or Alzheimer's disease in a subject, *e.g.*, a human subject, comprising administering to a subject a formulation comprising L-arginine and an HMG-CoA reductase inhibitor, thereby treating intermittent claudication intermittent claudication or Alzheimer's disease in a subject.

In yet another aspect, the invention features a method of treating intermittent claudication or Alzheimer's disease in a subject, *e.g.*, a human subject, comprising administering to a subject a formulation comprising L-arginine and a formulation comprising an HMG-CoA reductase inhibitor, thereby treating intermittent claudication or Alzheimer's disease in a subject. The formulation comprising L-arginine and the formulation comprising an HMG-CoA reductase inhibitor may be combined prior to administration to the subject. In one embodiment, the formulation comprising L-arginine and the formulation comprising an HMG-CoA reductase inhibitor are administered to the subject sequentially. In another embodiment, the formulation comprising L-arginine and the formulation comprising an HMG-CoA reductase inhibitor are administered to the subject concurrently.

In one embodiment, the HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, pravastatin, simvastatin, fluvastatin, dalvastatin, compactin, pitavastatin, mevastatin, fluindostatin, atorvastatin, cerivastatin, rosuvastatin, HR-780, BMY 22,089, BMY 22,566, SQ 33,600, GR 95,030, and CI 981, or a combination thereof.

In another embodiment, the formulation comprising L-arginine or the formulation comprising an HMG-CoA reductase inhibitor, or both, are administered intravenously, buccally, intracoronary, intramuscularly, topically, intranasally, rectally, sublingually, orally, subcutaneously, by patch, or by inhalation.

In yet another embodiment, the formulations further comprise at least one controlled release agent. In a further embodiment, either the formulation comprising L-arginine or the formulation comprising an HMG-CoA reductase inhibitor, or both, are in a controlled release formulation. The controlled release agent is present may be present in an amount sufficient to release the L-arginine over a period of about 4 hours to about 24 hours or over a period of about 8 hours to about 24 hours.

In another embodiment, the formulation comprising L-arginine contains L-arginine in an amount of about 100 mg to about 5 g or about 300 mg to about 700 mg. In another embodiment, the formulation comprising L-arginine contains L-arginine in an amount of about 60% to about 90% by weight of the formulation or about 65% to about 85% by weight of the formulation.

In yet a further embodiment, the formulation comprising L-arginine or the formulation comprising an HMG-CoA reductase inhibitor, or both, further comprise a filler, binder, excipient, lubricant, or a combination thereof.

In another embodiment, the formulations are administered prophylactically.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 is a graph depicting the release pattern of a formulation comprising L-arginine and simvastatin.

Detailed Description of the Invention

The present invention provides methods for the treatment or prevention of IC and AD. The present invention is based, at least in part, on the discovery that administering to a subject a formulation comprising an agonist of endothelial nitric oxide synthase (eNOS), such as an HMG-CoA reductase inhibitor, and a formulation comprising a precursor of NO, such as L-arginine, may be used to treat or prevent IC and AD in a

subject. The formulations may be administered to the subject either sequentially or concurrently. Alternatively, a single formulation comprising an agonist of NOS, (*e.g.*, an HMG-CoA reductase inhibitor), and a precursor of NO, (*e.g.*, L-arginine), may be used to treat or prevent IC and AD in a subject. In one embodiment, the formulations described herein may be used to slow the progression of AD in, for example, a minimally affected subject. In another embodiment, the formulations described herein may be used to delay the onset of the disease in, for example, populations at risk for development of AD.

Not to be limited by theory, the combination of L-arginine and an HMG-CoA reductase inhibitor has a beneficial effect in the treatment or prevention of IC and AD that is most likely due to excess L-arginine providing additional substrate for the NOS enzyme and NOS enzymatic activity being enhanced so that more L-arginine is converted to nitric oxide. Increased endothelial NO production results in enhanced NO dependent vasodilation, yielding improved blood flow and increased endothelial function, and can inhibit, reduce, or prevent tissue injury, stenosis, and other symptoms associated with IC and AD. In addition to improving endothelial function, administration of the formulations described herein may also decrease the amount of atherosclerotic plaque or prevent the formation of atherosclerotic plaque in arteries.

With respect to the treatment of AD in particular, administration of the formulations described herein to a subject suffering from AD results in increased cerebral blood flow and counterbalances the vascular stenosis associated with the decreasing cognitive status found in subjects suffering from AD. Similarly, administration of the formulations described herein to subjects with early stage AD could slow the progression of the disease in the subject by maintaining blood vessel elasticity, thereby delaying vascular stenosis. Administration of the formulations described herein may improve vascular functioning in the brain. Increased cerebral blood flow may improve short-term cognitive functioning and reduce long-term cognitive decline in patients with mild to severe Alzheimer's disease.

In one embodiment, the formulations used in the methods of the invention comprise at least one controlled release agent. In another embodiment, the L-arginine is slowly released into the system of a subject. The slow release of L-arginine creates a pharmacokinetic profile of L-arginine within the plasma that provides NOS with a

substantially constant supply of L-arginine needed for the production of NO. The formulations can slowly dissolve *in vivo* and release a substantially uniform amount of L-arginine over a time period to be therapeutically effective for a subject. In a further embodiment, the HMG-CoA reductase inhibitor is slowly released into the system of the subject. In a further embodiment, the production of NO is substantially uniform over a prolonged period of time.

Definitions

Before further description of the invention, certain terms employed in the specification, examples and claims are, for convenience, collected here.

As used herein, unless otherwise specified, the term "subject" includes mammals. The term "mammals" includes, but is not limited to, humans, dogs, cats, cattle, horses, pigs, and humans.

As used herein, "Alzheimer's disease" or "AD" is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, behavior and personality changes, and a decline in thinking abilities. It is characterized by two principle changes in brain tissues: development of amyloid plaques from incorrect processing of amyloid beta protein and formation of neurofibrillary tangles. These plaques and tangles develop only in the parts of the brain that control memory and retention of learned information. Other metabolic functions such as heartbeat, breathing, and digestion remain unaffected.

There are two types of AD: early onset and late onset. Early onset AD is rare (5-10% of AD cases), frequently occurs in families, and symptoms first appear before age 60. Late onset AD, the most common form of the disease, develops in people age 60 and over. Late onset AD lacks any familial association. Rather, modifications of the interactions of several distinct genes appear to significantly alter the formation of plaques and tangles in the brain. AD progresses slowly, *e.g.*, ranging from 3 to 18 years, with an average duration of 8 years. Ultimate death is not usually due to AD but results from some secondary illness such as pneumonia. One factor that might influence the severity and progression of Alzheimer's disease is cerebral blood flow (CBF). Two concurrent conditions suggest a possible association between CBF and cognitive decline in AD: the presence of beta-amyloid containing plaques, possibly impairing the microvascular circulation and the co-existence of atherosclerotic vascular disease.

As used herein, "intermittent claudication" or "IC" is a condition caused by underlying peripheral arterial disease (PAD), *e.g.*, atherosclerosis manifested by chronic arterial occlusion in the extremities, *e.g.*, the lower extremities. IC occurs when muscles of the extremities, *e.g.*, the lower extremities, do not receive required oxygen rich blood due to plaque buildup in the arteries of the extremities, especially during mobility, *e.g.*, exercise, including walking. As plaque builds up, blood flow decreases causing an altered metabolism in the oxygen-deprived tissues, which leads to IC. The primary symptoms of IC, which may occur primarily with mobility, *e.g.*, walking, include pain, ache, cramp, numbness, tightness, or fatigue in the extremities. IC is most common in the muscle groups of the calf, but may occur in the muscle groups of the foot, thigh, hip, or buttocks. IC may be worsened by walking rapidly or uphill. Symptoms of IC usually resolve with rest. However, with progression of the underlying disease, symptoms of IC begin at shorter and shorter distances walked, and ultimately, may even occur at rest. In severe cases, once blood flow is significantly reduced, symptoms may occur at rest, the muscles in the extremities begin to break down, and ulcers may appear. Bacteria may infect these ulcers and the resulting infection may spread into the blood stream leading to sepsis or into the muscles leading to gangrene.

As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent or formulation to a patient, or application or administration of a therapeutic agent or formulation to an isolated tissue from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, delaying onset of the disease or disorder, slowing the progression of the disease or disorder, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder.

As used herein, unless otherwise specified, the term "adjunctive administration" when used to describe the administration of two or more compounds to a subject means that the compounds, which may be administered by the same or different routes, are administered concurrently (*e.g.* as a mixture) or sequentially, such that the pharmacological effects of each overlap in time. As used herein, unless otherwise specified, when applied to the administration of at least two compounds, the term

"sequentially" means that the compounds are administered such that the pharmacological effects of each overlap in time.

As used herein, unless otherwise specified, the term "precursor of NO" includes any substrate precursor of native NO, *e.g.*, L-arginine.

The term "native NO" as used herein refers to nitric oxide that is produced through the bio-transformation of L-arginine or the L-arginine dependent pathway. The terms "endothelium derived relaxing factor (EDRF)" or "endothelium derived nitric oxide (EDNO)" may be used interchangeably with "native NO".

As used herein, unless otherwise specified, the term "L-arginine" or "arginine" includes, but is not limited to, L-arginine salts, isomers, pharmaceutically acceptable salts, or solvates, and combinations thereof. The term "L-arginine" or "arginine" also includes bioequivalents of L-arginine including, but not limited to, arginase inhibitors, lysine, citrulline, ornithine, and hydralazine or combinations thereof.

As used herein, unless otherwise specified, the term "agonist" or "agonist of eNOS or cNOS" refers to an agent which stimulates the bio-transformation of a substrate such as, for example, L-arginine to NO. An agonist of eNOS or cNOS includes, for example, an HMG-CoA reductase inhibitor. "HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A)" is the microsomal enzyme that catalyzes the rate limiting reaction in cholesterol biosynthesis. An "HMG-CoA reductase inhibitor" inhibits HMG-CoA reductase, and therefore inhibits the synthesis of cholesterol. HMG-CoA reductase inhibitors are also referred to as "statins."

There are a large number of compounds described in the art that have been obtained naturally or synthetically, which inhibit HMG-CoA reductase, and which form the category of agents useful for practicing the present invention. Traditionally these agents have been used to treat individuals with hypercholesterolemia. Examples include, without limitation, those which are commercially available, such as simvastatin (U.S. Pat. No. 4, 444,784), lovastatin (U.S. Pat. No. 4,231,938), pravastatin sodium (U.S. Pat. No. 4,346,227), fluvastatin (U.S. Pat. No. 4,739,073), atorvastatin (U.S. Pat. No. 5,273,995), cerivastatin, rosuvastatin, and numerous others such as compactin, dalvastatin, mevastatin, fluindostatin, pitavastatin, HR-780, GR-95030, CI 980, BMY 22089, BMY 22566, and those described in, for example, U.S. Pat. No. 5,622,985, U.S. Pat. No. 5,135,935, U.S. Pat. No. 5,356,896, U.S. Pat. No. 4,920,109, U.S. Pat. No.

5,286,895, U.S. Pat. No. 5,262,435, U.S. Pat. No. 5,260,332, U.S. Pat. No. 5,317,031, U.S. Pat. No. 5,283,256, U.S. Pat. No. 5,256,689, U.S. Pat. No. 5,182,298, U.S. Pat. No. 5,369,125, U.S. Pat. No. 5,302,604, U.S. Pat. No. 5,166,171, U.S. Pat. No. 5,202,327, U.S. Pat. No. 5,276,021, U.S. Pat. No. 5,196,440, U.S. Pat. No. 5,091,386, U.S. Pat. No. 5,091,378, U.S. Pat. No. 4,904,646, U.S. Pat. No. 5,385,932, U.S. Pat. No. 5,250,435, U.S. Pat. No. 5,132,312, U.S. Pat. No. 5,130,306, U.S. Pat. No. 5,116,870, U.S. Pat. No. 5,112,857, U.S. Pat. No. 5,102,911, U.S. Pat. No. 5,098,93 1, U.S. Pat. No. 5,081,136, U.S. Pat. No. 5,025,000, U.S. Pat. No. 5,021,453, U.S. Pat. No. 5,017,716, U.S. Pat. No. 5,001,144, U.S. Pat. No. 5,001,128, U.S. Pat. No. 4,997,837, U.S. Pat. No. 4,996,234, U.S. Pat. No. 4,994,494, U.S. Pat. No. 4,992,429, U.S. Pat. No. 4,970,231, U.S. Pat. No. 4,968,693, U.S. Pat. No. 4,963,538, U.S. Pat. No. 4,957,940, U.S. Pat. No. 4,950,675, U.S. Pat. No. 4,946,864, U.S. Pat. No. 4,946,860, U.S. Pat. No. 4,940,800, U.S. Pat. No. 4,940,727, U.S. Pat. No. 4,939,143, U.S. Pat. No. 4,929,620, U.S. Pat. No. 4,923,861, U.S. Pat. No. 4,906,657, U.S. Pat. No. 4,906,624 and U.S. Pat. No. 4,897,402, the disclosures of which patents are incorporated herein by reference. Any other member of the class of compounds that inhibits HMG-CoA reductase may be used in the methods of the invention. A combination of two or more HMG-CoA reductase inhibitors may also be used in the methods of the invention.

The term "eNOS activity", as used herein, means the ability of a cell to generate NO from the substrate L-arginine. Increased eNOS activity can be accomplished in a number of different ways. For example, an increase in the amount of eNOS protein or an increase in the activity of the protein (while maintaining a constant level of the protein) can result in increased "activity". An increase in the amount of protein available can result from increased transcription of the eNOS gene, increased translation of eNOS mRNA, increased stability of the eNOS mRNA, activation of eNOS (Kaesenmyer WH, *et al.* (1999) *J. Am. Coll. Cardiol.* 33(1):234), or a decrease in eNOS protein degradation.

The eNOS activity in a cell or in a tissue can be measured in a variety of different ways. A direct measure is to measure the amount of eNOS present. Another direct measure is to measure the amount of conversion of L-arginine to L-citrulline by eNOS or the amount of generation of nitric oxide by eNOS under particular conditions, such as the physiologic conditions of the tissue. The eNOS activity also can be measured

indirectly, for example by measuring mRNA half-life (an upstream indicator) or by a phenotypic response to the presence of NO (a downstream indicator). One phenotypic measurement employed in the art is measuring endothelial dependent relaxation in response to acetylcholine, which response is affected by eNOS activity. The level of NO present in a sample can be measured using a NO meter. All of the foregoing techniques are well known to those of ordinary skill in the art.

The methods of the present invention, by causing an increase in eNOS activity, permit not only the re-establishment of normal base-line levels of eNOS activity, but also allow increasing such activity above normal base-line levels. Normal base-line levels are the amounts of activity in a normal control group, controlled for age and having no symptoms which would indicate alteration of endothelial cell NOS activity (such as hypoxic conditions, hyperlipidemia and the like). The actual level then will depend upon the particular age group selected and the particular measure employed to assess activity. In abnormal circumstances, *e.g.* when a subject is afflicted with IC or AD, endothelial cell NOS activity is depressed below normal levels. According to the invention, using HMG-CoA reductase inhibitors can, not only restore normal base-line levels in such abnormal conditions, but can increase endothelial cell NOS activity far above normal base-line levels. Thus, "increasing activity" means any increase in endothelial cell NOS activity in the subject resulting from the treatment described herein, including, but not limited to, such activity as would be sufficient to restore normal base-line levels and such activity as would be sufficient to elevate the activity above normal base-line levels.

The term "carrier" refers to diluents, excipients and the like for use in preparing admixtures of a pharmaceutical composition.

As used herein, the term "dosage form" means a pharmaceutical composition that contains an appropriate amount of active ingredient for administration to a subject, *e.g.*, a patient either in single or multiple doses.

As used herein, unless otherwise indicated, the term "half-life" means the time taken to decrease the concentration of drug in the blood plasma of the organism by about one half from the drug concentration at the time of administration.

As used herein, unless otherwise specified, the term "immediate release" means that no extrinsic factors delay the *in vitro* release of one or more drugs.

As used herein, the terms "pharmaceutical composition" or "pharmaceutical formulation," used interchangeably herein, mean a composition that comprises pharmaceutically acceptable constituents.

As used herein, the term "pharmaceutically acceptable" means the type of formulation that would be reviewed and possibly approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

As used herein unless otherwise specified, the term "pharmaceutically acceptable carrier" means a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredient and which is not toxic to the subject to which it is administered. The use of such media and agents for pharmaceutically active formulations is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the formulations used in the methods of the invention is contemplated.

As used herein, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids and organic acids.

As used herein, unless otherwise specified, the term "sustained release" is defined as a prolonged release pattern of one or more drugs, such that the drugs are released over a period of time.

The term "substantially free" as used herein means that the composition contains a greater proportion of one isomer in relation to another isomer. In one embodiment the term "substantially free" as used herein means that the composition contains at least 90% by weight of one isomer and 10% by weight or less of a second isomer. These percentages are based on the total amount of isomers present in the composition. In another embodiment the term "substantially free" means that the composition contains at least 99% by weight of one isomer and 1% or less of a second isomer.

As used herein, the term "salt or complex" is used to describe a compound or composition comprising two or more chemical moieties that are associated by at least one type of interaction including, but not limited to, Van der Waals, ionic and/or hydrogen bonding. A salt or complex may exist as a solid or in a liquid.

As used herein, the term "weight percent" when used to describe the amount of a component within a formulation means the weight of the specified component based upon the weight of all components within the formulation.

Various aspects of the invention are described in further detail in the following subsections:

I. Formulations Used In Methods of Treatment or Prevention of IC and AD

The methods of the invention include methods of treating and preventing IC and AD in a subject, *e.g.*, a human, comprising administering to the subject a formulation comprising an HMG-CoA reductase inhibitor and a formulation comprising L-arginine, either concurrently or sequentially. Alternatively, a single formulation comprising L-arginine and an HMG-CoA reductase inhibitor is administered to a subject.

The formulations used in the methods of the invention may or may not comprise a controlled release agent. One embodiment of the invention encompasses formulations comprising L-arginine in a sustained release formulation, an HMG-CoA reductase inhibitor in a sustained release formulation, or both L-arginine and an HMG-CoA reductase inhibitor in a sustained release formulation. In one embodiment, the invention encompasses formulations comprising L-arginine which may be administered either concurrently or sequentially with at least one HMG-CoA reductase inhibitor wherein the formulation releases L-arginine in a substantially constant concentration over a prolonged period of time and the HMG-CoA reductase inhibitor is present in an immediate release formulation. In another embodiment, the invention encompasses formulations comprising L-arginine in a high concentration and in a sustained release formulation wherein the pharmacokinetic profile is zero order release kinetics (*i.e.*, linear release rate over time). The release characteristics of both classes of drugs may be modified to provide release patterns which allow for the adaptation of the combination into a once daily single unit dosage.

In one embodiment, the formulations used in the methods of the invention comprise L-arginine in a therapeutically effective amount, an HMG-CoA reductase inhibitor in a therapeutically effective amount, at least one controlled release agent, and at least one compression agent.

The formulations used in the methods of the invention may include additional ingredients necessary to modify the formulations for administration, preservation, esthetics and the like. In one embodiment, the formulations used in the methods of the invention also include cellulosics, polyacrylate and its derivatives.

In one embodiment of the invention, the formulations comprise L-arginine, solvates, or pharmaceutical salts thereof in an amount sufficient to allow for the continuous production of NO by eNOS without causing harmful side effects. In another embodiment, the formulations comprise L-arginine, solvates, or pharmaceutical salts thereof. USP grade L-arginine is commercially available from various sources including Sigma-Aldrich, 940 West St. Paul Avenue, Milwaukee, Wisconsin 53233-2681. Suitable arginine and arginine derivative compounds include, but are not limited to, arginine salts such as arginine HCl, arginine aspartate, or arginine nicotinate. Other arginine compounds or derivatives may be chosen from di-peptides which include arginine such as alanylarginine (ALA-ARG), valinyl-arginine (VAL-ARG), isoleucinyl-arginine (ISO-ARG), and leucinyl-arginine (LEU-ARG), and tri-peptides which include arginine such as argininyl-lysinyl-glutamic acid (ARG-LYS-GLU) and arginyl-glysyl-arginine (ARG-GLY-ARG). Typically, L-arginine, solvates, or pharmaceutical salts thereof are present in the formulations in an amount of about 50% to about 95% by weight of the formulation. In one embodiment, L-arginine, solvates, or pharmaceutical salts thereof are present in an amount of about 60% to about 90%. In another embodiment, L-arginine, solvates, clatharates, or pharmaceutical salts thereof are present in an amount of about 65% to about 85%. In yet another embodiment, L-arginine, solvates, clatharates, or pharmaceutical salts thereof are present in an amount of about 70% to about 84%. In a further embodiment, L-arginine, solvates, clatharates, or pharmaceutical salts thereof are present in an amount of about 73%.

In one embodiment, the HMG-CoA reductase inhibitor is present in an amount from about 0.01 mg to about 200 mg in the total formulation. The amount of HMG-CoA reductase inhibitor may vary based on the specific inhibitor present in the formulation, as some inhibitors are more efficacious than others. For example, BAYCOL® may be present in an amount of about 0.1 mg to about 0.8 mg per tablet, wherein ZOCOR® may be present in an amount of about 10 mg to about 80 mg per tablet. Those skilled in the

art will be able to determine a therapeutic amount based on the specific inhibitor employed.

The controlled release agent allows for the slow release of the L-arginine and/or the HMG-CoA reductase inhibitor over an extended period of time. For example, the controlled release agent may release L-arginine at a rate that will not cause concentration peaks or lows that would exacerbate side effects associated with high or low concentrations of L-arginine within the bloodstream. Controlled release agents suitable for the formulations used in the methods of the present invention include slowly hydrating agents. In other words, the controlled release agents form a substantially impermeable barrier to water such that water is slowly absorbed into the formulation thereby hydrating the formulation and subsequently releasing the active ingredient, *e.g.*, L-arginine at a rate substantially slower rate than a formulation without controlled release agents. Additionally, the controlled release agents are present in a particle size whereupon compaction or compression into a tablet, pill, capsule, or gelcap water slowly permeates into the structure.

In one embodiment, controlled release agents include, but are not limited to, cellulose ether products, polymethylmetacrylate, or polyvinylalcohol. In another embodiment, controlled release agents include methylcellulose, hydroxypropyl methylcellulose, or combinations thereof. In yet another embodiment, controlled release agents include, but are not limited to, hydroxypropyl methylcellulose. One suitable controlled release agent is commercially available from the Dow Chemical Company under the tradename METHOCEL®. In another embodiment, a controlled release agent is METHOCEL® K100 M CR Premium. The controlled release agent is typically present in an amount sufficient to release the active ingredient, *e.g.*, L-arginine over a desired period of time. In one embodiment, the controlled release agent is present in an amount of about 5% to about 40% by weight percent of the formulation. In another embodiment, the controlled release agent is present in an amount of about 10% to about 75%. In yet another embodiment, the controlled release agent is present in an amount of about 15% to about 50% by weight of the formulation.

The compression agent allows for the formulation to be shaped into a tablet, capsule, troche, gelcap, or other presentation for administration in solid form. In one embodiment, the compression agent allows the formulation to be shaped into a tablet,

capsule, troche, or gelcap. Compression agents are commercially available from The Dow Chemical Co. (Midland, MI 48674). Compression agents include, but are not limited to, Avicel, magnesium stearate, wax, gums, celleusics, stearate, or combinations thereof. In one embodiment, the compression agent is present in an amount of about 0.01% to about 5% by weight percent of the formulation. In another embodiment, the compression agent is present in an amount of about 0.5% to about 3%. In yet another embodiment, the compression agent is present in an amount of about 1% to about 2% by weight of the formulation.

In practical use, formulations used in the methods of the invention may comprise a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of the preparation desired for oral administration. In preparing the formulations for oral dosage form any of the usual pharmaceutical media may be employed. Usual pharmaceutical media include, but is not limited to, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like. Carriers include, but are not limited to, starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like. The most preferred oral solid preparations are tablets and gelcaps.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. Tablets or capsules may contain an L-arginine formulation and HMG-CoA reductase inhibitor formulation in the same tablet or capsule in different configurations. Configurations include, a two-part half and half tablet or capsule, one formulation surrounding a second, dispersion of one formulation in another, granules of both formulations intermixed, and the like. If desired, tablets or capsules may be coated by standard aqueous or nonaqueous techniques.

The formulations used in the methods of the present invention may also comprise other pharmaceutically acceptable ingredients, such as those commonly used in the art. *See*, Remington: the Science & Practice of Pharmacy, by Alfonso R. Gennaro, 20th ed., Williams & Wilkins, 2000. Additional ingredients used in the formulations used in the methods of the present invention include, but are not limited to, binders, fillers,

excipients, lubricants, disintegrants, diluents, carriers, stabilizing agents, coloring agents, flavoring agents, and combinations thereof.

Binders useful in the formulation include those commonly known to the skilled artisan. Binders include, but are not limited to, sugars, such as lactose, sucrose, glucose, dextrose, and molasses; natural and synthetic gums, such as acacia, guar gum, sodium alginate, extract of Irish moss, panwar gum, ghatti gum; other binders include methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, alginic acid, ethyl cellulose, microcrylstalline cellulose, zein, starch, gelatin, pregelatinzed starch, polyvinlypyrrolidone, and mixtures thereof.

Fillers useful in the formulation include those commonly known to the skilled artisan. Typical fillers include, but are not limited to, sugars such as lactose, sucrose, dextrose, mannitol, and sorbitol, whey, dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate, and mixtures thereof. Other fillers include, but are not limited to, cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone, and mixtures thereof.

Excipients can be added to increase the amount of solids present in the formulation. Among the excipients found useful for this purpose, often in combination, are sodium or potassium phosphates, calcium carbonate, calcium phosphate, sodium chloride, citric acid, tartaric acid, gelatin, and carbohydrates such as dextrose, sucrose, lactose, sorbitol, inositol, mannitol and dextran, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. In addition to those mentioned herein others are known to those skilled in the art.

Lubricants useful in the formulation include those commonly known to the skilled artisan. Typical lubricants include, but are not limited to, magnesium stearate, zinc stearate, calcium stearate, stearic acid, hydrogenated vegetable oils, sodium stearyl fumarate, glyceryl palmitostearate, glyceryl behenate, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, mineral oil, talc, and mixtures thereof.

Disintegrants include, but are not limited to, sodium starch glycolate, croscarmellose sodium, crospovidone, cross-linked polyvinyl pyrrolidone, corn starch, pregelatinized starch, microcrystalline cellulose, alginic acid, amberlite ion exchange

resins, polyvinylpyrrolidone, polysaccharides, sodium carboxymethylcellulose, agar, salts thereof such as sodium alginate, Primogel, and mixtures thereof.

Examples of suitable diluents include water, ethanol, polyols, vegetable oils, injectable organic esters such as ethyl oleate, and combinations thereof.

Pharmaceutically acceptable carriers are intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration including, but not limited to, sterile water, saline, buffered saline, dextrose solution, physiologically compatible buffers as Hank's or Ringer's solution, physiological saline, a mixture consisting of saline and glucose, heparinized sodium-citrate-citric acid-dextrose solution, and the like.

Formulations can also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be insured by various antibacterial and antifungal agents including, but not limited to, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents including, but not limited to, sugars, sodium chloride, and the like.

In another embodiment of the invention, the formulations may be "co-administered," *e.g.,* administered substantially simultaneously, with at least one other pharmaceutical agent, *e.g.,* a non-HMG-CoA reductase inhibitor. By substantially simultaneously, it is meant that the formulation of the invention is administered to the subject close enough in time with the administration of at least one pharmaceutical agent, whereby the two formulation and agent may exert an additive or even synergistic effect, *e.g.,* increasing NOS activity or on delivering a second agent to a tissue via increased blood flow.

Examples of categories of pharmaceutical agents include: adrenergic agent; adrenocortical steroid; adrenocortical suppressant; aldosterone antagonist; amino acid; ammonia detoxicant; anabolic; analeptic; analgesic; androgen; anesthetic; anorectic; antagonist; anterior pituitary suppressant; anthelmintic; anti-acne agent; anti-adrenergic; anti-allergic; anti-amebic; anti-androgen; anti-anemic; anti-anginal; anti-anxiety; anti-arthritic; anti-asthmatic; anti-atherosclerotic; antibacterial; anticholelithic; anticholelithogenic; anticholinergic; anticoagulant; anticoccidal; anticonvulsant;

antidepressant; antidiabetic; antidiarrheal; antidiuretic; anti-emetic; anti-epileptic; anti-estrogen; antifibrinolytic; antifungal; antiglaucoma agent; antihemophilic; antihemorrhagic; antihistamine; antihyperlipidemia; antihyperlipoproteinemic; antihypertensive; anti-infective; anti-inflammatory; antikeratinizing agent; antimalarial; antimicrobial; antimigraine; antimitotic; antimycotic, antinauseant, antineoplastic, antineutropenic, antiobessional agent; antiparasitic; antiparkinsonian; antiperistaltic, antipneumocystic; antiproliferative; antiprostatic hypertrophy; antiprotozoal; antipruritic; antipsychotic; antirheumatic; antischistosomal; antiseborrheic; antisecretory; antispasmodic; antithrombotic; antitussive; anti-ulcerative; anti-urolithic; antiviral; appetite suppressant; benign prostatic hyperplasia therapy agent; blood glucose regulator; bone resorption inhibitor; bronchodilator; carbonic anhydrase inhibitor; cardiac depressant; cardioprotectant; cardiotonic; cardiovascular agent; choleretic; cholinergic; cholinesterase deactivator; coccidiostat; cognition adjuvant; depressant; diuretic; dopaminergic agent; ectoparasiticide; emetic; enzyme inhibitor; estrogen; fibrinolytic; fluorescent agent; free oxygen radical scavenger; gastrointestinal motility effector; glucocorticoid; gonad-stimulating principle; hair growth stimulant; hemostatic; histamine H2 receptor antagonists; hormone; hypocholesterolemic; hypoglycemic; hypolipidemic; hypotensive; imaging agent; immunizing agent; immunomodulator; immunoregulator; immunostimulant; immunosuppressant; impotence therapy adjunct; keratolytic; LNRII agonist; liver disorder treatment; luteolysin; mental performance enhancer; mood regulator; mucolytic; mucosal protective agent; mydriatic; nasal decongestant; neuromuscular blocking agent; neuroprotective; NMDA antagonist; non-hormonal sterol derivative; oxytocic; plasminogen activator; platelet activating factor antagonist; platelet aggregation inhibitor; potentiator; progestin; prostaglandin; prostate growth inhibitor; prothyrotropin; psychotropic; radioactive agent; regulator; relaxant; repartitioning agent; scabicide; sclerosing agent; sedative; selective adenosine A1 antagonist; serotonin antagonist; serotonin inhibitor; serotonin receptor antagonist; steroid; stimulant; suppressant; symptomatic multiple sclerosis; synergist; thyroid hormone; thyroid inhibitor; thyromimetic; tranquilizer; treatment of cerebral ischemia; treatment of Paget's disease; treatment of unstable angina; uricosuric; vasoconstrictor; vasodilator; vulnerary; wound healing agent; or xanthine oxidase inhibitor.

Another example of a pharmaceutical agent includes angiotensin converting enzyme inhibitors (ACE inhibitors). ACE is an enzyme which catalyzes the conversion of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di and tri peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease. Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (U.S. Pat. No. 4,105,776) and zofenopril (U.S. Pat. No. 4,316,906), carboxyalkyl dipeptides such as enalapril (U.S. Pat. No. 4,374,829), lisinopril (U.S. Pat. No. 4,374,829), quinapril (U.S. Pat. No. 4,344,949), ramipril (US Pat. No. 4,587,258), and perindopril (U.S. Pat. No. 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (U.S. Pat. No. 4,512,924) and benazapril (U.S. Pat. No. 4,410,520), phosphinylalkanoyl prolines such as fosinopril (U.S. Pat. No. 4,337,201) and trandolopril. Estrogens upregulate NOS expression whereas ACE inhibitors do not affect expression, but instead influence the efficiency of the action of NOS on L-arginine. Thus, activity can be increased in a variety of ways. In general, activity is increased by the reductase inhibitors of the invention by increasing the amount of the active enzyme present in a cell versus the amount present in a cell absent treatment with the reductase inhibitors according to the invention.

The formulations used in the methods of the present invention can be formulated into pharmaceutically acceptable salts. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. Suitable non-toxic acids include inorganic and organic acids such as acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric acid, p-toluenesulfonic, and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, and sulfuric acids, and most particularly preferred is the hydrochloride salt.

Since the L-arginine used in the methods of the present invention is both basic and acidic, salts may be prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic and organic acids or inorganic and organic bases. Such salts may contain any of the following anions: acetate, benzensulfonate, benzoate, camphorsulfonate, citrate, fumarate, gluconate, hydrobromide, hydrochloride, lactate, maleate, mandelate, mucate, nitrate, pamoate, phosphate, succinate, sulfate, tartrate, and the like. Particularly preferred are benzensulfonate, hydrobromate, hydrochloride, and sulfate. Such salts may also contain the following cations: aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, and procaine.

A formulation used in the methods of the present invention is provided in a formulation that can provide therapeutically effective concentrations of L-arginine. The formulations release L-arginine substantially uniformly over a period of time greater than about four hours in a substantially linear or near zero order release essentially starting from ingestion, to provide substantially the same therapeutic efficacy for the drug as provided by the identical dosage of drug administered in divided doses. In one embodiment, the formulation releases L-arginine substantially uniformly over a period from about 4 hours to about 24 hours. In another embodiment, the formulation of the present invention releases L-arginine substantially uniformly over a period of about 8 hours to about 24 hours.

In another embodiment, a formulation used in the methods of the present invention will release L-arginine in a manner to provide a pharmacokinetic profile wherein the half- life ($T_{1/2}$) and the T_{max} are sufficient to maintain L-arginine at a substantially constant level. In other words, in one embodiment, a sustained release formulation used in the methods of the invention releases L-arginine such that a steady state of circulating L-arginine is achieved and remains constant. In one embodiment, the pharmacokinetic profile is such that $T_{1/2}$ is from about 4 hours to about 12 hours and the T_{max} is about 4 hours. In yet another embodiment, $T_{1/2}$ is from about 4 hours to about 8 hours and the T_{max} is about 4 hours.

In addition to the common dosage forms set out above, the formulations used in the methods of the present invention may also be administered by controlled release means and/or delivery devices such as those described in U.S. Pat. Nos.: 3,845,770;

3,916,899; 3,536,809; 3,598,123; and 4,008,719, the disclosures of which are hereby incorporated by reference.

II. Prophylactic Methods

In one aspect, the invention provides methods for preventing IC and AD in a subject by administering to a subject at risk for IC or AD a formulation comprising L-arginine along with a formulation comprising an HMG-CoA reductase inhibitor, either sequentially, or concurrently, or a single formulation comprising L-arginine along with an HMG-CoA reductase inhibitor. Subjects at risk for IC can be identified by, for example, a predisposition to atherosclerosis, symptoms of atherosclerosis, or by the presence of risk factors such as, for example, cigarette smoking, high blood pressure, diabetes, family history, genetic factors, and high cholesterol levels. Subjects at risk for AD can be identified by all of the above-listed risk factors, as well as advancing age and alcohol use. Administration of a formulation used in the methods of the invention as a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the onset of IC or AD, such that IC or AD is prevented, its progression slowed, or its onset delayed.

III. Methods of Administration

For any mode of administration, the actual amount of compound delivered, as well as the dosing schedule necessary to achieve the advantageous pharmacokinetic profiles described herein, will be depend, in part, on such factors as the bioavailability of the compound (and/or an active metabolite thereof), the disorder being treated, the desired therapeutic dose, and other factors that will be apparent to those of skill in the art. The actual amount delivered and dosing schedule can be readily determined by those of skill without undue experimentation by monitoring the blood plasma levels of administered compound and/or an active metabolite thereof, and adjusting the dosage or dosing schedule as necessary to achieve the desired pharmacokinetic profile.

The formulations used in the methods of the invention, as described herein, or pharmaceutically acceptable addition salts or hydrates thereof, can be delivered to a subject so as to avoid or reduce undesirable side effects according to the invention using a wide variety of routes or modes of administration. In one embodiment, the subject is

an animal. In another embodiment, the subject is a mammal. In yet another embodiment, the subject is a human. The most suitable route in any given case will depend on the nature and severity of the condition being treated. The preferred route of administration of the present invention is the oral route. The compositions may be conveniently presented in unit dosage form, and prepared by any of the methods well known in the art of pharmacy. Techniques and formulations for administering the compositions may be found in Remington: the Science & Practice of Pharmacy, by Alfonso R. Gennaro, 20th ed., Williams & Wilkins, 2000.

Pharmaceutical formulations used in the methods of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, troches, tablets, or gelcaps, each containing a predetermined amount of L-arginine and/or an HMG-CoA reductase inhibitor, as a powder, granules, a solution, or a suspension. Such formulations may be prepared by any of the methods of pharmacy, but all methods include the step of bringing into association L-arginine and/or an HMG-CoA reductase inhibitor with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing L-arginine and/or an HMG-CoA reductase inhibitor with liquid carriers or finely divided solid ingredients or both, and then, if necessary, shaping the product into the desired presentation.

For example, a tablet may be prepared by compression or molding, optionally, with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent, or combinations thereof. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. When the tablet comprises a controlled release L-arginine formulation and an HMG-CoA reductase inhibitor formulation, the tablet may have a core of slow release L-arginine formulation and a second outer cover of a formulation comprising at least one HMG-CoA reductase inhibitor. Alternatively, the tablet may comprise an L-arginine formulation, *e.g.*, a controlled release L-arginine formulation, and a HMG-CoA reductase inhibitor formulation sharing one surface.

When L-arginine is administered either sequentially or concurrently with HMG-CoA reductase inhibitors, each tablet, cachet, troche, or capsul contains from about 0.01 mg to about 200 mg of the HMG-CoA reductase inhibitors. The amount of an HMG-CoA reductase inhibitor will vary depending on the particular HMG-CoA reductase inhibitor utilized.

Alternatively, the formulation of the present invention is administered either sequentially or concurrently with at least one HMG-CoA reductase inhibitor in particular weight ratios. The range of weight ratios of HMG-CoA reductase inhibitors to L-arginine is dependent on the particular HMG-CoA reductase inhibitor. The HMG-CoA reductase inhibitor may be administered in an immediate release, intermediate release, or delayed release formulation.

The formulations used in the methods of the present invention may be administered to patients at least once a day, twice a day, *e.g.* once every 12 hours, or three times a day. Patients may be upward titrated from below to within this dose range to a satisfactory control of symptoms as appropriate.

For oral administration, the formulation can be formulated readily by combining L-arginine and/or an HMG-CoA reductase inhibitor with pharmaceutically acceptable carriers well known in the art. As discussed above, such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, troches, or gels for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in

admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, an excipient, a disintegrating agent, a lubricant, a glidant, a sweetening agent such as sucrose or saccharin, or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For buccal administration, the formulations may take the form of tablets or lozenges formulated in conventional manner.

Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous

administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the formulations described in the instant invention in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art,

and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

In one embodiment, the formulations used in the methods of the invention are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

IV. Effective Dosages

The formulations of the invention will generally be used in an amount effective to achieve the intended purpose, *e.g.*, to treat and prevent IC or AD. By therapeutically effective amount is meant an amount effective to treat, prevent, or ameliorate a disease or symptom related to a disease, *e.g.*, IC or AD, or prolong the survival of a subject being treated. Determination of a therapeutically effective amount is well within the capabilities of those skilled in that art, especially in light of the detailed disclosure provided herein.

Pharmaceutical formulations suitable for use with the present invention include formulations wherein L-arginine and/or an HMG-CoA reductase inhibitor are contained in a therapeutically effective amount, *i.e.*, an amount effective to achieve the intended purpose. In general, an effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, produces the desired response. This may involve only slowing the progression of the disease temporarily. In another embodiment,

it involves halting the progression of the disease permanently or delaying the onset of or preventing the disease or condition from occurring. The effect of the dosage on any particular disease can be monitored by routine methods. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner.

Generally, doses of active compounds would be from about 0.01 mg/kg per day to 1000 mg/kg per day. In one embodiment, it is expected that doses ranging from 50-500 mg/kg will be suitable. In another embodiment, administration is orally and in one or several administrations per day.

Of course, the actual amount of L-arginine and/or an HMG-CoA reductase inhibitor will depend on, among other things, the condition of the subject, and the weight and metabolism of the subject. For example, when administered to a subject suffering from IC or AD, a tablet, pill, dragee, capsule, gelcap, troche, or capsule, will contain an amount of L-arginine and/or an HMG-CoA reductase inhibitor effective to, *inter alia*, ameliorate the harmful effects of insufficient blood flow to normal tissue, *i.e.*, prevent the development of or alleviate the existing symptoms of, or prolong the survival of, the subject being treated. Determination of an effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

For any formulation described herein the therapeutically effective amount can be initially estimated from 0.25 to 4 g/daily.

Therapeutically effective amounts for use in humans can also be estimated from animal models. For example, a dose for humans can be formulated to achieve a concentration found to be effective in animals.

A therapeutically effective dose can also be estimated from human pharmacokinetic data. While not intending to be bound by any particular theory, it is believed that efficacy is related to a subject's total exposure to an applied dose of administered drug, and/or an active metabolite thereof, as determined by measuring the area under the blood concentration-time curve (AUC). Thus, a dose administered according to the methods of the invention that has an AUC of administered compound

(and/or an active metabolite thereof) within about 50% of the AUC of a dose known to be effective for the indication being treated is expected to be effective. A dose that has an AUC of administered compound (and/or an active metabolite thereof) within about 70%, 80% or even 90% or more of the AUC of a known effective dose is preferred.

Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD50/ED50. Formulations which exhibit large therapeutic indices are preferred. While formulations that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such formulations to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. In one embodiment, the dosage of such formulations of the instant invention lies within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any formulation used in the therapeutic or prophylactic methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Adjusting the dose to achieve maximal efficacy in subjects based on the methods described above, particularly on the blood concentration and duration of administered compound and/or its active metabolites is well within the capabilities of the ordinarily skilled artisan.

For other modes of administration, dosage amount and interval can be adjusted individually to provide effective plasma and/or tissue levels of the L-arginine, and/or an active metabolite thereof, according to the pharmacokinetic profiles described herein, as previously described.

V. Pharmacogenomics

With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics," as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype").

Thus, another aspect of the invention provides methods for tailoring an subject's prophylactic or therapeutic treatment with the formulations used in the methods of the present invention according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects. Accordingly, in conjunction with the therapeutic and prophylactic methods of the invention, pharmacogenomic (*i.e.*, the study of the relationship between a subject's genotype and that subject's response to a foreign compound or drug) methods are also provided.

Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a pharmaceutical formulation as described herein for treatment and prevention of IC or AD.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp.Pharmacol. Physiol.*

23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate aminopeptidase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach" can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known, all common variants of that gene can be fairly easily

identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and the cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling" can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a formulation used in the methods of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of a subject. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and, thus, enhance therapeutic or prophylactic efficiency when treating a subject suffering from IC and AD with an pharmaceutical formation as described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: Tablet Formulation 1

L-Arginine (250 g) was placed in a mixer and as it was slowly mixed (with low stirring (100 RPM)), Eudragit water dispersion (RS 30 D) (100 g) was added to form a wet mass. The wet mass was passed through 18-20 sieves and allowed to dry at 50°C for 24 hours. The resulting dry L-arginine granulars (250 g) were dry mixed with methylcellulose (Methocel, Dow, K100 M CR, 84 g) and magnesium stearate (3 g) to form a blend. The resulting blend was compressed into tablets using 7/16 concave punches.

EXAMPLE 2: Tablet Formulation 2

L-Arginine (250 g) was placed in a mixer and as it was slowly mixed, methylcellulose (Methocel, Dow, K100 MCR, 84 g) and magnesium stearate (3 g) was added. The resulting blend was compressed into tablets using 7/16 concave punches.

EXAMPLE 3: Gelcap Formulation 1

L-Arginine (250 g) was placed in a mixer and as it was slowly mixed (with low stirring), Eudragit dispersion (RS 30 D) (100 g) was added to form a wet mass. The wet mass was passed through 18-20 sieves and allowed to dry at 50°C for 24 hours. The resulting dry L-arginine granulars (250 g) were dry mixed with methylcellulose (Methocel, Dow, K100 M CR, 84 g) and magnesium stearate (3 g) to form a blend. The resulting blend was placed into 00 gel capsules.

EXAMPLE 4: Gelcap Formulation 2

L-Arginine (250 g) was placed in a mixer and as it was slowly mixed, methylcellulose (Methocel, Dow, K100 M CR, 84 g) and magnesium stearate (3 g) was added. The resulting blend was placed into 00 gel capsules.

EXAMPLE 5: Tablet Formulation 3

L-Arginine (250 g) and methylcellulose (Methocel, Dow, K100 MCR, 50 g) were mixed and homogenized using a Kitchen Aid® mixer on low speed for 10 minutes to form a dry blend. To the dry blend, a dispersion of Eudragit (RS 30 D, 115 g) was added in 5 g increments until the mass was homogeneously wet. The wet mass was passed through a 12 mesh sieve followed by a 20 mesh sieve and subsequently, allowed to dry at 30°C for 24 hours until the moisture content was 1% by weight. The resulting dry L-arginine granulars were dry-mixed with magnesium stearate (7 g) and then compressed, using Beta Manesy press, into tablets using 7/16 concave punches.

EXAMPLE 6: Manufacturing of a Sustained Release Tablet

L-Arginine and methocel are mixed in the GP-1 for 5 ± 1 minutes at 100 RPM. Eudragit RS 30D dispersion is then added with the impeller running at 200 RPM and a pressure of 1.5 bar. The mixture is granulated for 1 minute at 200 RPM. The granulation is then dried in the MP-1 Fluid Bed Granulator at 45°C inlet temperature with an air flow of 100 CMH for 3 ± 1 hours to approximately 2% moisture content. The dried granules are then milled using a Comil 197S with size 55R screen and round impeller at 90% speed. In an 8 Qt. V-Blender, magnesium stearate is added to the milled granules and mixed for 2 minutes. The material is then compressed into tablets with a target weight of 682.5mg ± 5 to highest possible hardness using a Beta Manesty Press with 7/16" standard concave tooling. The tablets are hand packaged at 60 tablets per bottle in 75 cc HDPE Bottles.

EXAMPLE 7: Evaluation of pharmacokinetics of L-arginine

A randomized, four-way crossover design to evaluate the pharmacokinetics of L-arginine sustained release tablets versus immediate release capsules was conducted on 14 healthy adult volunteers under fasting conditions.

The study goals were to determine the pharmacokinetic parameters of L-arginine sustained release.

Based on the p-values from the two-tailed paired t-test performed on each pharmacokinetic parameters, there was a statistically significant difference between treatments for C_{max} and T_{max}. As expected, sustained release L-arginine tablets had a lower C_{max} (14.9 ug/mL versus 24.1 ug/mL) and a longer T_{max} (4.4 h versus 1.4 h) compared with the immediate release capsules.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. A method of treating intermittent claudication or Alzheimer's disease in a subject comprising administering to a subject a formulation comprising a precursor of NO and a formulation comprising an agonist of eNOS, thereby treating intermittent claudication or Alzheimer's disease in a subject.

2. The method of claim 1, wherein said precursor of NO is L-arginine and said agonist of eNOS is an HMG-CoA reductase inhibitor.

3. A method of treating intermittent claudication or Alzheimer's disease in a subject comprising administering to a subject a formulation comprising L-arginine and an HMG-CoA reductase inhibitor, thereby treating intermittent claudication or Alzheimer's disease in a subject.

4. A method of treating intermittent claudication or Alzheimer's disease in a subject comprising administering to a subject a formulation comprising L-arginine and a formulation comprising an HMG-CoA reductase inhibitor, thereby treating intermittent claudication or Alzheimer's disease in a subject.

5. The method of claim 3 or 4, wherein said HMG-CoA reductase inhibitor is selected from the group consisting of lovastatin, pravastatin, simvastatin, fluvastatin, dalvastatin, compactin, pitavastatin, mevastatin, fluindostatin, atorvastatin, cerivastatin, rosuvastatin, HR-780, BMY 22,089, BMY 22,566, SQ 33,600, GR 95,030, and CI 981, or a combination thereof.

6. The method of claim 3 or 4, wherein said subject is a human.

7. The method of claim 4, wherein said formulation comprising L-arginine and said formulation comprising an HMG-CoA reductase inhibitor are combined prior to administration to said subject.

8. The method of claim 4, wherein said formulation comprising L-arginine and said formulation comprising an HMG-CoA reductase inhibitor are administered to said subject sequentially.

9. The method of claim 4, wherein said formulation comprising L-arginine and said formulation comprising an HMG-CoA reductase inhibitor are administered to said subject concurrently.

10. The method of claim 4, wherein either said formulation comprising L-arginine or said formulation comprising an HMG-CoA reductase inhibitor, or both, are in tablet form.

11. The method of claim 4, wherein said formulation comprising L-arginine or said formulation comprising an HMG-CoA reductase inhibitor, or both, are administered intravenously, buccally, intracoronary, intramuscularly, topically, intranasally, rectally, sublingually, orally, subcutaneously, by patch, or by inhalation.

12. The method of claim 11, wherein one or both formulations are administered orally.

13. The method of claim 3 , wherein said formulation further comprises at least one controlled release agent.

14. The method of claim 3 or 4, wherein either said formulation comprising L-arginine or said formulation comprising an HMG-CoA reductase inhibitor, or both, are in a controlled release formulation.

15. The method of claim 13 or 14, wherein said controlled release agent is present in an amount sufficient to release the L-arginine over a period of about 4 hours to about 24 hours.

16. The method of claim 15, wherein said controlled release agent is present in an amount sufficient to release L-arginine over a period of about 8 hours to about 24 hours.

17. The method of claim 3 or 4, wherein said formulation comprising L-arginine contains L-arginine in an amount of about 100 mg to about 5 g.

18. The method of claim 17, wherein said formulation comprising L-arginine contains L-arginine in an amount of about 300 mg to about 700 mg.

19. The method of claim 3 or 4, wherein said formulation comprising L-arginine contains L-arginine in an amount of about 60% to about 90% by weight of the formulation.

20. The method of claim 19, wherein said formulation comprising L-arginine contains L-arginine in an amount of about 65% to about 85% by weight of the formulation.

21. The method of claim 3 or 4, wherein said formulation comprising L-arginine and an HMG-CoA reductase inhibitor, said formulation comprising L-arginine or said formulation comprising an HMG-CoA reductase inhibitor, further comprise a filler, binder, excipient, lubricant, or a combination thereof.

22. The method of claim 3 or 4, wherein said formulations are administered prophylactically.

23. The method of claim 3 or 4, wherein the progression of Alzheimer's disease is slowed.

24. The method of claim 3 or 4, wherein the onset of Alzheimer's disease is delayed.

Figure 1 of 1

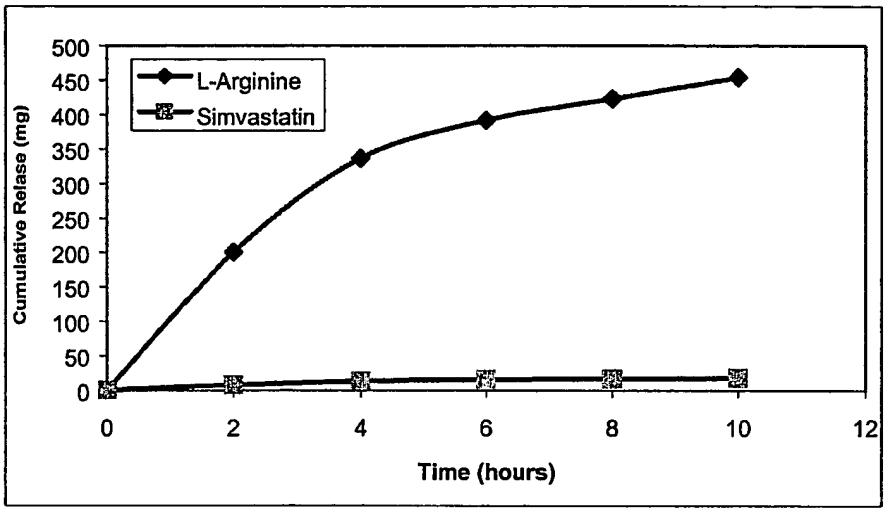

FIGURE 1

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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF INTERMITTENT CLAUDICATION OR ALZHEIMER'S DISEASE

(57) Abstract: The present invention provides methods for the treatment and prevention of intermittent claudication or Alzheimer's disease in a subject, comprising administering to a subject a formulation comprising a precursor of NO, e.g., L-arginine and a formulation comprising an agonist of eNOS, e.g., and HMG-CoA reductase inhibitor, or a formulation comprising both L-arginine and an HMG-CoA reductase inhibitor. The invention further provides that the formulations used to treat or prevent intermittent claudication or Alzheimer's disease contain at least one controlled release agent. In a further embodiment, the production of NO is substantially uniform over a prolonged period of time.

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,E	US 6,537,987 B1 (HAMANAKA et al.) 25 March 2003 (25.03.2003), column 2, lines 26-33, column 17, lines 52-64 and column 36, lines 28-56.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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